THE SPERMATOGENESIS OF THE CADDIS-FLY (PLATYPHYLAX DESIGNATUS WALKER).

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The numerous works that have appeared in recent years on the spermatogenesis of insects have covered practically every order and in some orders, such as the Hemiptera, many of the important families. There still remain, however, a number of the smaller orders which have not been investigated, and there is always the possibility that these may contain new or especially favorable structures. The Trichoptera is one of these small orders. The Neuroptera and Lepidoptera to which it is believed to be most nearly related have been investigated by Miss McGill (8) and by Toyama (15) and Henking (2). The small size of the larvæ and the difficulty of dissecting out the very young tests has probably deterred investigators from attacking this group when larger, better known and more easily obtainable forms were to be had in the Hemiptera and Orthoptera.

I shall not go into an extensive discussion here of the general literature of spermatogenesis. *Platyphylax* has in the main the same development that has been described for all insect forms. There are certain points in which it is different and the literature on them will be discussed in connection with my own observations.

The only paper in which the spermatogenesis of the Trichoptera is discussed at all is in that of Lubben (3). Lubben was more interested in the external morphology and the development of the testes than he was in the cytological details, but he observed a number of facts well worth noticing. The spermagonial cells arise out of the original genital cells by division. They grow into large cells which appear, united with two cyst epithelial cells. The origin of these epithelial cells of the cysts was not clear to him. Inside these epithelial cells now arises a mass of cells by division. Then by two more divisions the spermatids are produced; these have sharply bounded nuclei with clear plasma around them. The spermatids increase in length and the whole

complex of spermatozoa takes on the form of a long-drawn out cylinder. The nuclei are all arranged at one end, pointing one way, while the body of the sperm fills the remaining space. These he calls spermatocysts.

The formation of the free spermatozoids does not seem to occur either in the free or the enclosed larvæ. At the time of pupation there are only spermatocysts. In the pupa there results along with growth and the further differentiation of the spermatozoids the resorption of the walls of the spermatocysts. In the mature pupa the spermatozoa are free and lie in thick knots in each division of the testes.

The caddis-flies, as has long been known, pass practically their entire life in the larval and pupal stage. Vorheis (17), who has followed the life-history of *Platyphylax designatus*, has found that the eggs are laid in April and that the larvæ appear about two weeks later. During summer and fall the larvæ grow, and from November to January more and more larvæ are found. The period of pupation begins about the middle of February and is indicated by larger irregular stones being attached to the anterior end of the sand cases "while some are attached to the lower surface of the large rocks by a mass of silk at the anterior end." For a few days after the closing of the case the larvæ remain inactive but unchanged, before becoming pupæ.

The caddis-fly larvæ offer the advantage that while the life-history just sketched is gone through with at about the time indicated, larvæ of almost any size may be still obtained up until January and February. The material was obtained during three years; the first year from about the first of December to the first of February; the second year during the month of June, and the third year in May. The spermagonial and reduction division occur in the larval stage, so the half-grown larvæ of a length of 6–10 mm. was the material in which it was found. The sperms are apparently all formed at or soon after pupation.

In the older specimens the testes were dissected out, but in order to get the very young spermagonia it was necessary to section the entire abdomen.

Practically all the material was fixed in Fleming's weaker solution although sublimate-acetic was used on some abdomens

with very good results. The sections were stained in Heidenhain's iron alum hæmatoxylin or in Flemming's triple stain.

THE TESTES.

The testes, as Vorheis (17) has already noted, occur in the fourth and fifth segment of the abdomen. They are small five lobed structures usually surrounded by a mass of fat.

These five lobes or follicles are about equal size but of various shapes. In general they are somewhat wedge-shaped, the thinner part of the wedge being, of course, toward the point of attachment of the organ (Fig. 13). In longitudinal section about fifty cysts appear in each follicle (Fig. 14), the larger ones at its smaller end.

Each follicle has its own wall and the flattened nuclei of these wall cells frequently appear in the sections. In addition each cyst is provided with a wall. The youngest cells are farthest from the point of attachment of the testes. In one section, as shown in Fig. 14, cysts containing spiremes for the first division, tetrads, first and second divisions, transformations of the spermatids, and fully developed sperms occur in the order named across the section. The young cysts show in section, 6–8 spermatocytes; the older ones, where the cells are smaller, due to the two divisions and to the growth of the cysts, show 12–18. There is no definite zonation of spiremes, first divisions, second divisions, etc., such as Wilcox (19) has figured in *Caloptemus femur-rubrum*, but the general tendency is for the cysts containing the younger cells to be further away from the smaller end of the follicles. The fact that there is no definite zonation of successively older spermatocytes makes it at first confusing in picking out the sequence of development. The difference in size and number of them in the different cysts however makes this possible after a little observation. The spermatids in developing in the cysts, as is usual, have their heads all pointing in one direction as they lie side by side.

THE SPERMAGONIAL CELLS.

As the early history of the spermagonial cells does not seem to have been worked out on many species of insects a rather full description of that process will be given here. In this insect the

primary and secondary spermagonial cells are sharply distinguished in their division stages while the development of the cysts with their surrounding epithelial cells is almost diagrammatic in its clearness.

There seems to be a general agreement in the literature that each of the cysts that lie in the follicles of the testes has its origin from a single germ-cell. This conception seems to have originated in the work of Vallette St. George (12) on the testes of *Rana temporaria*. He found that if he teased apart the parenchyma of the testes, groups of cells, that he called spermatocysts, would drop out. These structures had walls of their own in which were imbedded one or two nuclei. St. George believed that each of these cysts arose from a single cell, one of his "Ursamenzellen." These would correspond to the last of what is now known as the primary spermagonial cells. By the division of these cells there was produced the spermacysts containing the spermagonia; or as now called, the secondary spermagonia.

Montgomery (10) describes the cysts in *Pentatoma* but did not work out their origin. The connective tissue network of the young testes contains, he believes, in each mesh a single spermagonium or at least only a few spermagonia. These cells divide and the cells produced by their division are surrounded by a cyst-membrane derived from an extension of the connective tissue investment of the follicle. The germ-cells and the cells of the cyst walls have then a different origin.

Several others, among them Henking, Paulmier and McGregor, are mentioned by Sutton (14) as having noted the arrangement into cysts and the cyst walls but none of them seems to have followed their development.

Sutton (14) in studying the spermagonial divisions in *Brachystola magna* also worked out fairly completely the development of the cysts. A cyst membrane with nuclei in it is formed even in the two-celled stage of the secondary spermagonia. The cysts assume a roughly pyramidal shape, the cells inside it largely dividing tangentially to the surface of the cyst. All the cells in one cyst seem to divide simultaneously producing by means of about eight divisions, 256 cells. The cysts are not attached to the walls of the follicles.

In all of the species so far described, however, the cysts lie so closely appressed against each other and against the follicular wall that it is impossible to decide certainly as to the identity of the cyst walls. In all of them too the number of cells seems to be large. In these respects *Platyphylax* offers a much better opportunity for deciding the question of cyst wall and cell number in a cyst.

The earliest stage of the reproductive organ that I was able to be sure was the testes was that shown in Fig. 1. At this stage there has already appeared the lobing into five divisions that is so characteristic of the mature organ. The cells composing it are apparently all of about the same size; there being at this time no visible differentiation into germ-cells and cyst epithelial cells, if such does occur. The nuclei of these cells are a little larger than those in other parts of the body. The distribution of chromatin in these nuclei is characteristic of that in all parts of the body, the pieces being of rather large size and of a rather regular number instead of having the form of fine granules. Sometimes these pieces of chromatin are in the form of bodies which might well be taken for split chromosomes, while others have the diamond shape characteristic of the tetrads with which they might readily be confounded (Fig. 3). Divisions are rather rare at this time and growth is apparently rather slow. The division figures observed were of typical mitotic type. The peculiar part that the nucleolus plays in this division will be discussed under the reduction divisions as in them it is much larger and more readily followed. All that is necessary to say here is that it seems to change its spherical shape, becomes elongated and apparently forms a chromosome.

In the testes next advanced in the stage of development the primary spermagonial cells occupy only about a third of the space while the remainder of it is filled with the secondary spermagonial cells in various stages of division to form the mature cysts can be followed at every stage.

The testes have grown considerably. The five divisions are fully formed; they have acquired distinct cellular boundaries; and the original secondary spermagonial cells, instead of being rather closely packed in the testes, are now lying with consider-

able free space between them. This free space is what makes the task of following them, and their boundaries, so easy. About two thirds of the primary cells, spermagonia, or "Ursamenzellen" as they are variously called, have divided to form groups of 2, 4, 8, 16 or 32 cells while the other third is undivided.

A closer examination of these undivided cells will now show that in addition to the larger germ-cell there is lying closely appressed against it a smaller kidney-shaped nucleus (Fig. 4). The chromatin in both nuclei is distributed in the form of little flecks rather regular in size and number and united by strands.

The origin of this nucleus that lies in the epithelium of the cyst is not clear. There is no such differentiation in the cells in the testes at the preceding stage. If these epithelial cells were in the testes at that time it was impossible to distinguish them. It may be that it is an epithelial cell that has made its way in some fashion into the interior of the testes and there surrounded the germ-cell. It is a difficult question to decide and one on which my material gives little evidence.

The further development of the cysts of the follicle can be readily followed in the two- (Fig. 5), four- (Fig. 6), eight- (Fig. 7), sixteen- and thirty-two-celled (Fig. 8) stages. The cell-walls are difficult to distinguish at these stages as the plasma membrane which is all that surrounds the cells is very thin and the cells are pressed tightly against each other. The same difficulty was noted by St. George (12) who found, however, that he could distinguish the walls in material fixed in "quick acting reagents." At any of these stages the nucleus of the cyst epithelium will show. In the older cysts there appear quite frequently to be two such nuclei, but in the younger stages at least I have observed only one. These nuclei do not seem to divide.

The divisions in the secondary spermagonial cells are very regular, all the cells in one follicle dividing at one time (Fig. 9). All these divisions are typically mitotic. Sutton (14) found that the division spindles were largely tangential to the walls of the cyst, owing to pressure in those directions. This caused the cyst to grow in length. As the cysts of the caddis-fly are spherical, the divisions occur in equal numbers in all planes. It can be readily determined that there are regularily 32 cells in each cyst of the

follicles, which indicates, of course, that five divisions have occurred.

After the last division, the cells of these mature cysts grow and the nucleus becomes much larger in proportion to the size of the cell. Fig. 8 shows the size of the cyst immediately after it has acquired its full number of cells and Fig. 10 shows a cyst after the growth period in which the cells are in the spireme for the reduction divisions. There are therefore two growth periods: one after the last primary spermagonial division and the second before the reduction divisions.

The Reduction Divisions.

After the last spermagonial divisions the growth is apparently very rapid. The nucleus is very large, occupying almost the entire cell and the chromatin is in the form of regular little pieces (Fig. 11). The remains of the spindle of the last spermagonial division lies near the nucleus in the form of an ovoid body, the nebenkern.

Apparently the first stage in division shows the little pieces of chromatin drawn out until they resemble chromosomes while the nucleolus becomes oval or spindle-shaped (Fig. 12). The pieces of chromatin become united by a slender connection and seem to spin out and lose their identity. The nucleolus in the meantime becomes more and more elongated (Fig. 15).

The chromatin now seems to go into a long slender spireme, the mass of the threads lying at one side of the nucleus and occupying about one half of it. This is apparently the stage of synapsis. The strands are very fine and delicate and as the nuclei are not so very large, it is impossible to make out any pairing of the threads, if such occurs. The nucleolus lies as a long drawn out body either in this tangle of the threads or on its surface. Occasional loops stick up out of the denser clump but they are too small to follow or to make much out of. After these stages, which are apparently short as they are not numerous, the chromatin again fills the entire cell in the form of the spireme. This spireme gradually becomes shorter and thicker. This stage is a very long one as is shown by the fact that in sections where any divisions at all occur, about half of the cells are in this stage.

It may be that the stage of synapsis is much shortened in the caddis-fly and that of the spireme is correspondingly lengthened.

The spireme now breaks up into chromosomes and these lie in the nucleus as long slender paired bodies (Fig. 22). The chromosomes now come to have the peculiar shapes such as X's, Y's, twisted figures, etc., characteristic of the stage of the reduction division (Fig. 23). These soon shorten into the tetrads (Fig. 24). The tetrads take on the typical lozenge form and sometimes show an opening in their center. The manner in which these bodies arrange themselves on the spindle could not be definitely determined as they are small in size and the four segments of the lozenge are all of about the same length.

The metaphase shows a sharp pointed spindle with its extremities in a centrosome just inside the plasma membrane (Fig. 25). The telophase of this division shows the chromosomes pulled about two-thirds of the way back to the centrosome and the nuclei still connected by the remains of the central spindle (Fig. 26). The centrosome at this time is still apparently divided and the rays from it extend down to the cluster of chromosomes.

The second division, following almost immediately, has little to distinguish it in the metaphase from the first except in its size and in the size of its chromosomes (Fig. 29). In the telophase of this division the centrosome was not to be found—just what had become of it has not been ascertained. The remains of the central spindle is the most conspicuous feature of this stage. The chromosomes seem to spread out at once, as soon as a nuclear membrane is formed, and make the ordinary reticulated network of a resting nucleus (Fig. 34).

There is a period of growth after the reconstruction of the nucleus, such as Paulmier (11) and other authors have described, followed by a stage in which the nucleus shrinks. Even before the chromosomes are entirely distributed and while they are still present as little pieces of chromatin (Fig. 34), the cell begins to lengthen and the axial filament to form. In fact the long drawn out form of the cell which it has from the last division does not seem to change but passes over at once to the young sperm. The transition stage takes a long time for its completion and any number of transition stages can be found. The chromatin col-

lects more or less to the outside of the nucleus, forming a hollow sphere, just inside the nuclear wall. The remains of the spindle lie near the nucleus as an oval body in a clear zone. This body apparently divides as Baumgartner (1) has described and a part of it seems to pass around the nucleus. Soon after these stages shown in Fig. 34 the nuclei become very sensitive to the fixing reagents and as a consequence practically all of them have collapsed. This would probably be the stages when the chromatin is in the form of a tube with a very small lumen. The fully developed sperm is shown in Fig. 36.

The details given above are in the main the same as have been described many times for animal spermatogenesis. In the action of some parts of the cell mechanism there is always quite a little variation and it is to these parts that particular attention will be paid. The two structures to be especially noted are the centrosome and the "chromatin nucleolus."

The Centrosome.

It is impossible to locate the centrosome except when it is occupying a position at the end of the spindle. At any other place it is impossible to say whether a certain dark staining body is a centrosome or not. Bodies appear near the nucleus where the centrosome should be and have all the characteristics of centrosomes, being darkly staining little granules surrounded by a clear space and frequently with what appear to be rays running up to them, though these latter are very faint and small, but the multiplicity of such bodies makes it impossible to trace the history of the centrosome with any certainty for any distance or even to be sure that such a centre is present at all times. At the ends of the spindle, its position is of course always easy to determine. It usually first appears so as to be definitely recognized at the tetrad stage as a dark body on the plasma membrane. It appears as a very small black granule on which the fibres terminate and lies just inside the plasma membrane and apparently attached to it. It is most conspicuous during the metaphase when the sharp pointed spindle ends in this little granule at the plasma membrane. It may be, of course, that here it is not a body at all but only the common point of attachment of the fibres of the

central spindle and of the aster. Whatever the origin and nature of the centrosomes may be at this time it is at least something that will take a stain and that has a definite location. After the second division, at which time it lies at the ends of the spindle again, it seems to disappear until a dark staining granule appears at one side of the nucleus from which the axial filament seems to be growing out. I have not traced the centrosome around to discover whether the two are identical or not but from the results on other animals it undoubtedly is. From this stage on then, it would form the middle piece of the sperm.

The Chromatin Nucleolus.

McClung (4) has described in the germ-cells of certain grasshoppers a body which he calls the accessory chromosome. Previous to this discovery of this body the "chromatin nucleolus" had been described by Montgomery (10) in the Hemiptera. More recently the discussion of heterotypic chromosomes has been given special importance by the papers of Stevens (13) and Wilson (20, 21) especially in connection with the theory of sex-determinants.

The divisions in the nuclei of *Platyphylax* show a body which, while it seems to have something in common with these described structures, is in other respects quite different. Its behavior has been reserved for this separate discussion.

The various changes undergone by this body have been followed to some extent both in the spermagonial and reduction divisions. As the cells, and consequently this chromatin nucleolus, are larger in the reductions divisions it will be described there first. The nucleus of the young spermatocytes contains a nucleolus that stains typically both in the triple and the iron haematoxylin. This body is either spherical or ovoid in shape. In the preparatory stages of division it begins to lengthen and become spindle shaped. It frequently lies twisted over on itself or is spoon-shaped at this time (Fig. 15).

In a later stage when the chromatin has gone into the synaptic condition this body seems as a rule to be somewhat smaller in diameter as though it were spun out as the other chromatin has been (Fig. 16). It does not lie among the chromatin strands, but

as a rule rests outside the chromatin mass which at this time occupies about half the nucleus. At this time the body seems to be a part of the spireme. In the next stage when the chromatin comes out of synapsis this body appears as a part of the much thickened spireme thread (Figs. 17–21). It is quite large at this time and much resembles a nucleolus in its intense staining reactions but it is spindle shaped and from each end runs out the continuation of the spireme thread. In the triple stain at this time it still takes the safranin color. A still closer examination shows that the threads leading up to this body are double and the body itself is divided into two halves by a longitudinal furrow. It lies at this time, when seen in side view, as a flat spindle-shaped figure immediately pressed against the nuclear wall. It now resembles very much the accessory chromosome as drawn by McClung (6) in his Fig. 2, except that he at this period discovered no break and the body that he drew was proportionally considerably larger. He describes and figures a stage (Fig. 5) where this body goes into a spireme of its own but no split was observed in this separate thread. Considering the subsequent behavior of this body—the formation of a tetrad and of chromosomes, such a split is to be expected. A split was to be observed in this chromatin nucleolus of *Platyphylax*; its spireme is a part of the spireme formed from the remainder of the chromatin. The split in it becomes more marked (Fig. 19) and the body finally opens out as a lozenge-shaped tetrad (Fig. 21). At this time the other chromosomes have not yet formed, although the longitudinal split has taken place. In some cases it looks as though the transverse splits have already occurred, but the thread still remains intact with this body as a part of it. This black staining tetrad is one of the most conspicuous parts of the nucleus at this time (Fig. 21).

The other chromosomes are now formed and assume the peculiar shapes characteristic of them at the time before they form the tetrads. This body is still recognizable at this time on account of its regular lozenge shape while the others are in the form of X's, Y's and various other twisted shapes (Fig. 23). At the next stage, however, when all the chromosomes have become tetrads this body is indistinguishable from them (Fig. 24).

There is no evidence that it has disappeared as the nucleolus usually does; it seems simply to have become a tetrad. The elaborate formation and dividing of the tetrad would argue against this disappearance also. This chromatin nucleolus can be traced no further. During the equatorial plate stage of division the chromosomes all lie in one plane and it is impossible to identify any particular one as the transformed nucleolus. Neither does any one lag behind in divisions in the metaphase nor in the movement toward the poles in anaphase (Figs. 26 and 32). If this body forms a chromosome, as it undoubtedly does, that chromosome behaves exactly like all the others.

The number of chromosomes is of great interest here if this is a true accessory chromosome. According to either the McClung or the Wilson type of an accessory chromosome, or the Wilson type of a heterotypic one, there should appear an odd number of chromosomes plus this additional one; or as McClung has found in *Orchesticus* sixteen chromosomes and the accessory one.

In all the counts made in *Platyphylax*, however, the number of chromosomes for both the reduction divisions was found to be thirty. This is the result of repeated trials. These countings are as easy to make as of dots on a piece of paper (Figs. 27, 28, 30) as the polar plate views are numerous and the chromosomes are short. There is some variation in size in the chromosomes in polar view but it is impossible to pick out one of them as the special structure that has been followed.[1]

It will be seen from this description that while this body resembles the accessory chromosome of McClung in many respects still it differs from it in one very essential one. It apparently forms a tetrad that divides in both divisions and so each sperm would receive one fourth of it. This would make it impossible for it to serve as a sex-determinant, for all the sperms would receive a part of it, and not half of them, as would happen if we credit the observations of McClung or of Miss Wallace (18). This body in that case could not be a sex-determinant.

[1]In cutting abdomens to get the development of the testes I have cut and stained as many, or more, females than I have males. The divisions in the former can be readily observed here as they are much larger than in the testes. The nucleolus undergoes a similar lengthening out, and then forms a part of the spireme. Marshall (9) in his paper on the development of the ovary also shows several figures that strongly suggests this.

Voinov (16) has described in the divisions preceding sperm formation in a beetle (*Cybister roeselii*) a body which very much resembles in its appearance the one under discussion. He, however, did not observe it forming a chromosome tetrad, nor did he follow it during the nuclear divisions except to figure a small darkly staining body lying outside the nucleus in the cytoplasm, which he believes to be the same. It may be that this body might form a tetrad as in *Platyphylax*.

Heidenhain in "Plasma und Zelle" approves Flemming's opinion that the nucleolus is always surrounded by a thin layer of true chromatin. Some appearance such as those shown in Fig. 2 would seem to suggest at least that there might be a ring of some other material around the nucleolus but the structure is so small that it is impossible to say certainly. If this were true, however, the changes described would only mean that the nucleolus loses its form during the divisions and becomes pulled out and split, between the chromatin strands surrounding it.[1]

At any rate whatever this structure in *Platyphylax* may be, whether "accessory chromosome" or "chromatin nucleolus," some disposition of it must be made in discussing these aberrant chromosomes in the nuclei of insects.

This work was done under the direction of Prof. W. S. Marshall, of the University of Wisconsin, to whom my thanks are due not only for assistance with methods and literature but also for quite a portion of the material from which the sections were made.

SUMMARY.

1. The development of the follicular cysts can be readily followed in this insect. Each cyst contains 32 cells derived by 5 divisions from a primary spermagonial cell and enclosed in a membrane containing one or two nuclei.

2. The reduced chromosome number is always 30; the somatic number is probably 60 from a count in the oogonial divisions.

3. The centrosome is only to be followed from the tetrad stage to the anaphase but probably forms the middle piece of the sperms.

[1] A count of the somatic number of chromosomes in the oögonial division gave 55–60. The exceedingly large number makes counting difficult and not very accurate in these divisions.

4. The nucleolus of the spermatocyte seems to form a tetrad which becomes one of the thirty of the reduced number.

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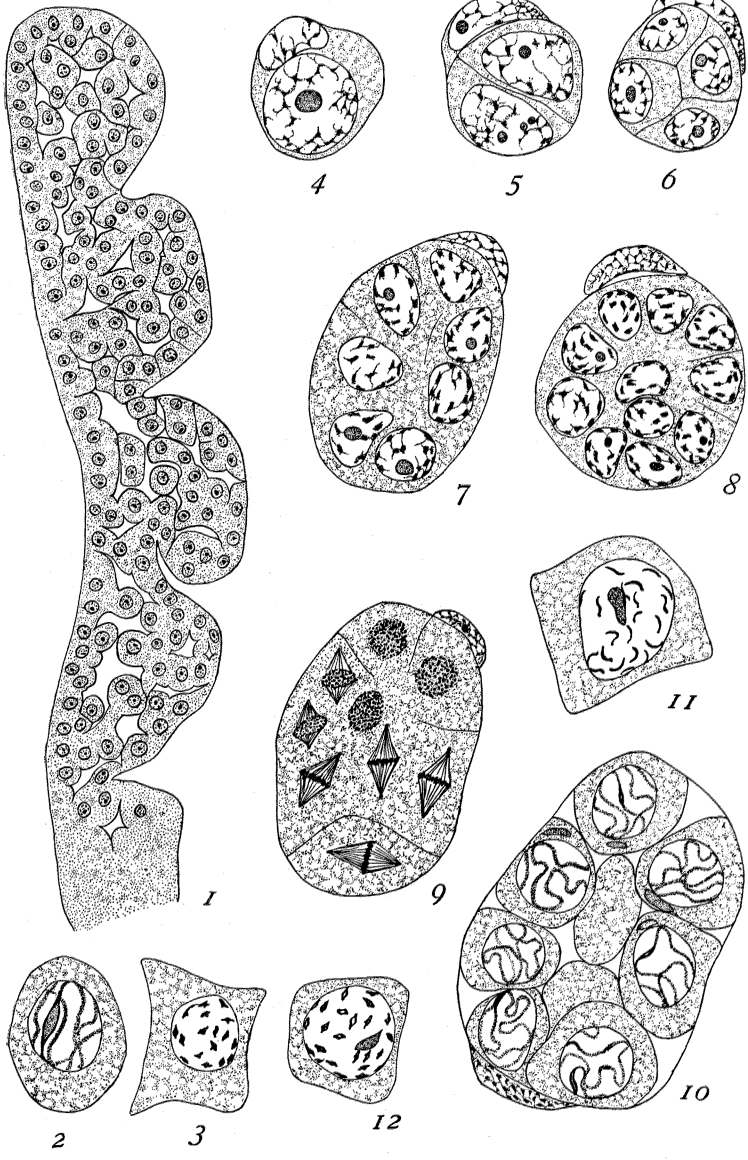
Explanation of Plate I.

Magnification. (1) 80; (13) 25; (14) 80; all the others are magnified about 1,200 diameters except No. 20 which is about 1,800.

1. Longitudinal section of very young testes showing primary spermagonia cells.
2. Spermagonial cell in spireme.
3. Resting spermagonial cell.
4. One-celled stage of cyst showing epithelial nucleus lying alongside of it.
5. Two-celled stage of cyst.
6. Four-celled stage.
7. Sixteen-celled stage.
8. Thirty-two-celled stage.
9. Sixteen-celled stage dividing.
10. Mature cyst with the nuclei all in the spireme stage; after the growth period.
11. Young spermatocyte just leaving the resting condition.
12. Young spermatocyte showing lengthening of the nucleole.



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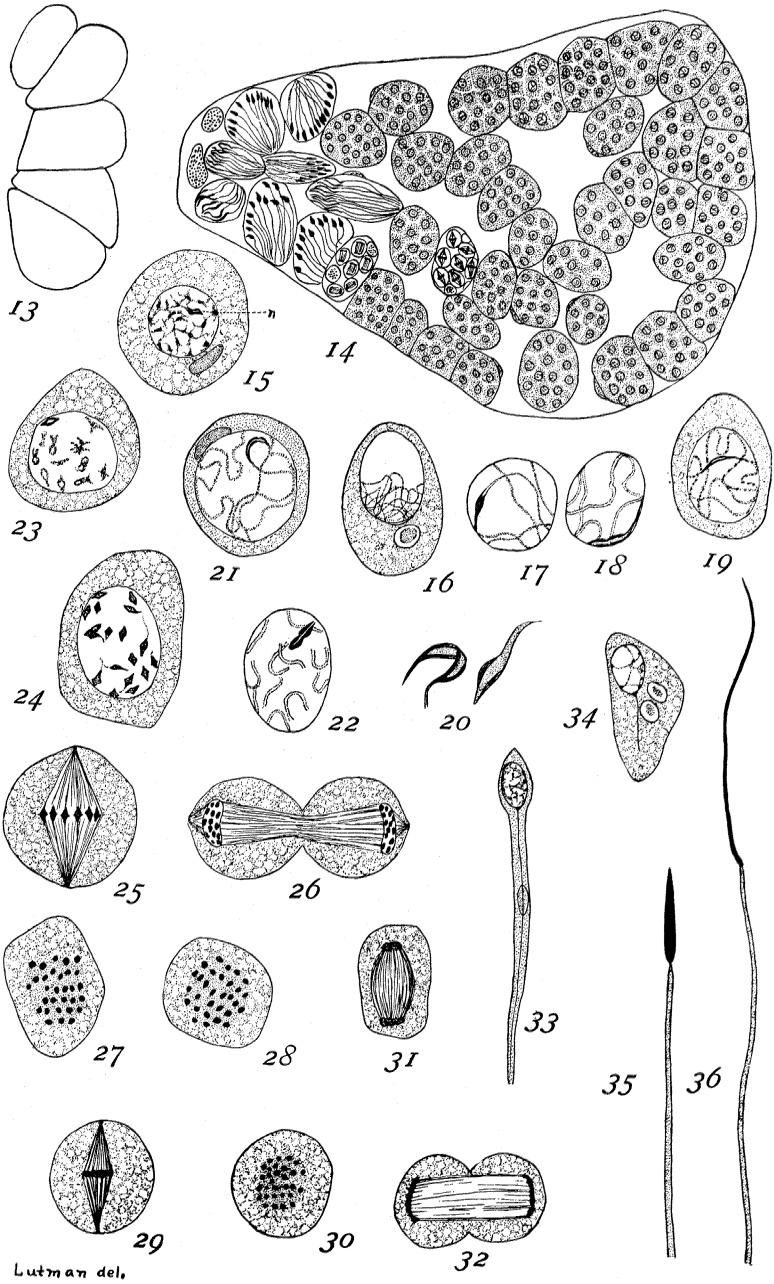
Explanation of Plate II.

13. Longitudinal diagram of the entire mature testes.
14. Section of one of the follicles showing size, shape, and distribution of the cysts.
15. Formation of the spireme in a spermatocyte.
16. Synapsis.
17. Nucleus showing nucleolus on spireme.
18. Nucleus showing nucleolus on spireme.
19. Entire cell showing same.
20. Enlarged views of the nucleolus at this same stage showing the split.
21. Chromosomes formed by the transverse split.
22. Chromosomes lying free in the nucleus; also the split nucleolus.
23. Chromosomes forming the tetrads; nucleolus at *n*.
24. Tetrads.
25. Metaphase, first division.
26. Anaphase, first division.
27–28. Polar view of the equatorial plate.
29. Metaphase, second division.
30. Polar view, equatorial plate.
31–32. Anaphase, second division.
33–35. Transformation stages of the spermatid.
36. Sperm.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